WHO Working Group on HIV Incidence Assays
Annual Meeting 18-24 August 2011
Atlanta, Georgia, USA
1. Background

Since 1998 different serological tests for testing new HIV infections have been in use or under development. However, these serological approaches have been challenged by various obstacles: i) variability of the immune response among HIV-1 infected individuals and the impact of antiretroviral therapy and late-stage AIDS immunosuppression leading to inaccuracy in identifying persons with recent infection; ii) variation in the window period for different HIV-1 subtypes or populations; iii) difficulty in the standardization of quality control measures; iv) the continued availability of commercial assays; and v) the complexity and high cost of the assays. Because of these factors, the use of HIV incidence assays has been the subject of some debate and controversy.

In 2008 WHO established a Working Group on HIV Incidence Assays to look into these issues. This group is made up of epidemiologists, laboratory specialists and public health officials, and has worked to standardize terminology in the areas of assay calibration and validation. Several meetings to advance the agenda have been held and copies of reports are available on the HIV Incidence Working Group web page: http://www.who.int/diagnostics_laboratory-links/hiv_incidence_assay/en/. These meetings have been successful in bringing together a wider group of assay users, in particular from countries affected by the epidemic who may consider using HIV incidence assays in the future with key experts in the field of applying laboratory-based methods for estimating HIV incidence. The importance of HIV incidence as a key indicator of national programme success or failure was highlighted and it was clear that Ministries of Health need to be aware of the complexities of producing estimates based on data generated by the currently available assays.

Moreover, the new UNAIDS and WHO 5-year strategies aim to reduce HIV incidence and even though there is no clear consensus on how to measure incidence in all countries, HIV incidence assays can provide a good tool, especially for high burden countries. Furthermore, in the most recent report by the Institute of Medicine in 2010 (Preparing for the future of HIV/AIDS in Africa) that advises PEPFAR programs, one of key recommendations is: **Measure incidence: African countries, with the support of donors, should develop and implement cost-effective methods for accurately measuring the level of and change in HIV/AIDS incidence to enable better planning and evaluation of HIV/AIDS prevention programs.**

In collaboration with the Centers for Disease Control and Prevention (CDC), Family Health International 360 (FHI 360), South African Centre for Epidemiological Modeling and Analysis (SACEMA,) and other institutions, the HIV incidence Working Group has produced a guidance document on "**How to estimate HIV incidence at the population level using HIV incidence assays in cross-sectional studies**".

The last meeting of the steering committee of the Working Group on HIV Incidence was held in Geneva in May 2010. Following this meeting in 2010 the Bill and Melinda Gates Foundation (BMGF) adjudicated a grant proposal to the Health Protection Agency (UK)/ Blood Systems Research Institute (US) to continue laboratory work on the existing HIV incidence assays. This approved
proposal has five main objectives and aims to validate existing and future HIV incidence assays, comparing results from existing assays with direct incidence measurements, and to identify the key parameters to allow for correct interpretation of the laboratory results. The grant is for two years and work is ongoing.

In the last meeting held by the WHO HIV incidence working group it was agreed that two additional documents on HIV incidence assays should be developed: a field validation protocol and the pathway recommendations for developers. Discussions were held with CDC/US to organize the 2011 working group meeting and the cost was shared by both organizations. CDC agreed as an alternative to hold the meeting in Atlanta.

This report follows the agenda.

2. Objectives of the Meeting

- Provide recommendations on statistical issues around incidence estimation using tests for recent infection
- Provide an update on specimen repositories and cross-assay comparisons
- Provide update on in-house and field evaluations of new and existing tests for recent infection
- Provide update on new biomarkers for estimating HIV incidence using tests for recent infection
- Review WHO guidance documents for development, evaluations, and use of tests for recent infection to estimate HIV incidence
- Discuss the future role and agenda of the WHO HIV Incidence Assay Working Group

3. Methods of work

The agenda and presenters are listed in Annex 1. The first two days of the meeting were dedicated to the discussion of statistical approaches to estimate the mean duration of recency and statistical issues around estimating HIV incidence using cross-sectional surveys.

During the early session on day 3 with the larger group, the recommendations from statisticians were presented and other general issues were discussed as described in the Agenda of the meeting.
4. Statistics Subcommittee Meeting 18-19 August 2011

4.1 Session A: Approaches for estimating the mean duration of recency

The overall goal of this session was to evaluate the current methods used to estimate incidence and discuss their limitations. Speakers presented analyses using existing data and highlighted statistical challenges, particularly related to estimation of the mean duration of recency (MDR) period and the false recent rate.

Several methods were used for estimating the MDR using data from different cohorts (Amsterdam, Ethiopia, Kenya, Trinidad) from which specimens were collected and tested with 4 assays—Avidity Index (AI), BED, Limited Antigen Avidity (LAg), and the less sensitive (LS) antibody assay. There were 396 observations in total; known limitations with BED were highlighted. Two estimation methods were presented: survival analysis and Bayesian modeling. Much of the discussion focused on the advantages and limitations of survival versus Bayesian approaches. There was some discussion on whether optimum cut-off values for the assays can be chosen based on where minimum coefficient of variation is reached.

Four additional statistical methods were presented: Singly and Doubly interval-Censored Likelihood, Non-Parametric Survival with Multiple Imputation, and Linear Mixed Effects Regression. Using these methods, BED and Avidity data from 115 subjects with HIV subtype B, who had 536 observations in total, were analyzed. Several shortcomings for these estimation procedures were discussed:

- Use of interval-censored likelihood methods does not utilize all of the available information about quantitative changes in bioassay measures after infection.
- Use of linear mixed effects models are sensitive to unusual data, e.g., zero or negative slopes which may result from inadequate follow-up or presence of long-term non-progressors.
- Survival methods may result in biased MDR estimates due to the presence of data from long-term non-progressors because of censoring after the latest event (crossing the bioassay threshold for distinguishing recent from long-term infection).

There was discussion about the limitations of the present formula to calculate incidence and introduction of a new formula with a term to reflect an arbitrary time (T) since infection as the period for which the parameters for number of recent infections and MDR are assessed. It was agreed that accurate estimation of mean recency duration is necessary for accurate incidence estimation.

After a robust discussion on the estimation of the mean duration of recency, the group decided that the formula should be changed to add a parameter (T) to reflect the mean duration of recency. Some discussion also focused on sample sizes necessary for incidence estimation and whether parameters could be agreed upon to include in the guidance.
The new parameter $T$ was defined as: *The time an individual spends in assay/algorithm-defined recency state up to a fixed time, $T$*

**Advantages:**
- Provides unambiguous definition
- Decouples short-term and long-term time scale
- Requires panel data only “a little" beyond $T$ to estimate distribution/mean
- Accounts for those who vacillate in and out of recency state
- Requires only slight changes in incidence formulae
- Stabilizes estimates of mean given by different statistical methods
- $[0, T]$ should cover dynamic range of assay (and not much more)
- Choice has implications for requirements for panel data
- For currently available assays, $T \approx 1$ year is probably appropriate
- Different specimen sets needed to estimate false recent rate (FRR), but these are generally easier to acquire

However, several issues were raised about the new parameter $T$ and estimation of MDR and incidence:

(a) How do we choose $T$?
(b) How many specimens within each clade are needed for incidence estimation?
(c) How should we account for clade to clade variability?
(d) What should we do about the early plateau for bioassay levels? Is it important?
(e) Is BED extrapolated to a background level inaccurately accounting for the early plateau after infection?
(f) Is variance calculated correctly?
(g) Misclassification of specimens from persons on antiretrovirals should be considered
(h) Should we separate responders (those subjects who reach the bioassay cutoff value) from non-responders (those who are not observed to ever reach the cutoff value)? Or are non-responders accurately accounted for using the new incidence algorithm and estimation of MDR <$T$? What about persons who are false longstanding?

The discussions in the afternoon were based on the morning presentations—approaches for estimating the mean duration of recency and the new parameter $T$. The participants discussed a new approach for estimating the mean window period and other various approaches in methodology. It was agreed that the goal is to find an assay that will have a long mean duration of recency and a minimal false recent rate. Simulations will need to be conducted to determine specifications for the specimen panels needed to validate these parameters.

Some of the issues raised during the discussion were:

i. New definition: mean time spent recently infected for less than time $T$ (each individual estimated to have mean recency period as a percentage of $T$)
   - Small changes in formula
   - Two independent approaches yielded similar results
   - Simplifies formula for incidence
   - Time $T$ needs to consider the characteristics of the assay
   - Requires bioassay with minimal FRR
   - More research needed on what occurs after time $T$
• Minimally acceptable number of specimens needed between \( T_0 \) and Time \( T \) needs to be determined
• Bioassay threshold for distinguishing between long-standing and recent infection needs to be determined

ii. Simulations/panels
• Panels should consist of approximately 100 specimens
• Last negative and first positive specimens and dates should be included
• Time between last negative to first positive should be no longer than 3 months
• Need to consider missing values
• Recommendations regarding the number and frequency of specimens will be determined after simulation studies are conducted
• Need funding to run simulations

iii. Further discussion needed
• Description of a panel
• Acceptable methods for determining window period
• Methods need to be assay-based

Regarding the simulation panels it was suggested that as starting point—
• 100 subjects per clade were needed
• Known seroconversion interval \( \leq 3 \) months
• The specimens collected at first positive test and at 3-month intervals thereafter (up to “a little” beyond \( T \))
• Enroll seroconverters for panel follow-up from a variety of on-going studies
• Could begin to construct panels from existing specimens

The main conclusions and recommendations at the end of the session were:

➢ Re-estimation of mean duration of recency based upon predefined definition of \( T \) is needed
➢ The time period of recent infection should be set as \( T=\text{time positive and alive} \leq 1 \text{ year} \)
➢ Specimen data should be collected just beyond time \( T \) (for example, if time \( T \) is one year, the period of data collection would be for 18 months)
➢ Estimates may not be the most accurate, but they are adequate for practical purposes
➢ Separate studies are required to estimate the false recent rate (FRR) but more details in designing these studies are needed
➢ An ideal panel would have approximately 100 subjects with the time period recommended previously (for example, \( = 18 \) months)
➢ The frequency and timing of data collection is to be determined
Due to outside statistical considerations in sampling for behavioral surveillance surveys or other complex sampling surveys, another group should be formed to create recommendations in estimates for recent infections

Specimens from persons on ARVs or with low CD4 counts that test false recent perhaps should not be excluded from the FRR calculations

Combinations of assays should be considered

In the face of incomplete data and other issues, simulation work is recommended to gain a better understanding of the behavior of procedures to estimate incidence

Simulation of combination assays should also be considered

No attempt should be made to discourage/dissuade investigators from using any tool (e.g., incidence and sample size calculators) at their disposal; however—

- the Working group will maintain a reference tool with version control
- SACEMA will update the current tool—
  - Incorporate new duration of recency concept
  - Allow viewing of multiple analyses simultaneously
  - Increase guidance (warning messages) for users

SACEMA will produce simulation panels to share with different statisticians to test the validity of the assumptions

4.2 Session B: Sample size calculators and considerations for sample sizes

This session reviewed two Excel spreadsheets that can be used to calculate sample size for HIV Incidence studies. One was developed by SACEMA and the other by CDC. Each of them presented an overview of their sample size calculators.

The Excel file developed by SACEMA contains the following tabs:

- Prevalence/incidence calculator-- calculates point estimates and CIs for Incidence and annual risk of infection
- Hazard ratio calculator
- A p-value calculator-- gives a p-value for the difference of incidence between two studies
- Sample size calculator-- calculates sample size required for a specified CoV using assay characteristics and background incidence.
- Statistical power calculator-- calculates the power to detect a reduction in incidence using two surveys (excludes parameter uncertainty). The user must input incidence, prevalence, sample sizes and an alpha.

This tool is the one recommended in the new guidelines for estimating HIV incidence at the population level using HIV incidence assays.
The ideal HIV incidence test should have a longer MDR and a low FRR. New tests have the lower FRRs but not the longer MDR. If a test has a very low FRR, it might not be possible to get enough false recent results to generate a good estimate of the FRR. This may result in non-normal CI/likelihoods. But this isn't something to worry about, since in this scenario the FRR is so small that it does not need to be calculated precisely. The incidence differences/hazard ratios don’t require a known MDR.

CDC presented the tool used to calculate power and sample sizes needed for incidence estimation. This Excel workbook was developed by CDC in order to fill the need to do multiple calculations easily. The Excel workbook has four modules:

- Power determination given two sample sizes
- Sample size calculations: sizes are proportional to the coefficients of variation of incidence
- Sample size calculation: sizes are equal
- Power calculation considering CV of FRR and mean recency period

Use of the CDC tool has shown that the CoV of recency period has a large effect while the CoV of the FRR has a small effect. The incidence calculator workbook is available on PEPFAR.net, and can be accessed by all those with a user ID and password.

CDC presented a second calculator, currently in development, which is used to determine the sample sizes needed to detect a specified change in incidence between two surveys based on the desired power (e.g., 80%). Alternatively, it can be used to calculate the power to detect a specified change in incidence between two surveys based on sample sizes.

The main conclusions and recommendations at the end of the session were:

- People can use whatever tools they desire.
- But the working group should recommend one of the workbooks as the standard that can be used to check calculations (SACEMA tool is recommended by the WHO guidance document).
- HIV incidence testing should not be included in the majority of most-at-risk-population (Key Populations) studies at this point, because of small sample sizes and an insufficient number of positive specimens.
- Calculating HIV incidence with respondent-driven sampling (RDS) weighting and design effect has not been worked out.
- Incidence tests are needed with longer RITA durations, not just lower FRRs.
- **Update SACEMA’s tools, which are available online at www.SACEMA.com:**
  - Complete coverage for input parameter uncertainty
  - Allow multiple scenarios to be calculated at once
  - More guidance, caveat remarks added to make it easier to use
  - Add remarks on definitions
  - Add the ability to vary the two sample sizes to calculate power to detect differences in incidence
- **Update CDC tools, which are available online at www.PEPFAR.net**
4.3 Session C: Incidence estimation and statistical testing

In this session, CDC presented the work being done to calculate HIV incidence in the US used the BED assay. CDC has published in 2006 and 2010 the estimated number of new infections and its geographical, age and sex distribution. The methods used for those HIV incidence estimates were presented.

A limited number of States both report HIV cases and use the BED assay. About 60% of the US population lives in areas that participate. At participating sites, about 40% of those newly diagnosed with HIV were tested for recency. Anyone diagnosed with AIDS (CD4 less than 200-or opportunistic infection within 6 months after HIV diagnosis) was classified as not recent regardless of test result. These individuals and anyone on ARTs should not have been tested using the BED assay, but if they were, their result was changed to long term (for AIDS cases) or missing (for cases on ART) regardless of their result on the test.

BED is assumed to be a reliable test for HIV-1 subtype B. An FRR in the US was not available nor used to adjust for incidence estimates.

For the rest of the country where HIV case reporting data was not available a stratified extrapolation approach was used. This approach contributed to:

- Identifying cases diagnosed in a calendar year from states with incidence surveillance data
- Imputing missing data
- Stratifying cases by sex, race/ethnicity, age group and transmission category
- There were 68 different strata
  - Estimated incidence in each stratum
  - Adjust estimates for reporting delay
  - Extrapolate results from states with incidence data to other states.

Some of the key assumptions on which these estimates are based were:
- Conditional on the observed variables, previous test and BED results are missing at random
- Information on previous test or T is accurate
- BED mean recency period distribution is well defined
- Timing of HIV testing is independent of HIV infection
  - Testing behavior has not significantly changed
  - Incidence is relatively constant (recent about 2 years)
  - Incidence ratio of HIV to AIDS in the incidence states is similar to the ratios in the other states
  - Ratio of NEW HIV diagnoses to NEW AIDS diagnosis

Issues and recommendations regarding HIV incidence estimates:

- Confidence intervals should probably be even wider because of sources of uncertainty that were not accounted for in the calculations
Members of the Technical Working Group (TWG) expressed the need to look more carefully at how the incidence was calculated before they could accurately comment on the methodology.

In the future, the FRR should be accounted for in these types of calculations. However this becomes difficult because unlike other types of studies, some HIV+ individuals are excluded from testing because they are known AIDS cases. What really needs to be accounted for is the FRR among those who are tested. This may be different than the FRR among the total population of HIV+ individuals, and it could be difficult to determine.

 Sessions 1-3: August 22, 2011

5. 1 Session 1: Overview of Incidence Assays and Incidence Estimation Methodologies: Challenges Facing the Field

This session was an overview of the ideal incidence assay and the status of currently available assays, which measure various immunologic stages of infection. The presenters described the advantages and disadvantages of each assay type and also the challenges involved in incidence estimation.

Remarkable progress has been made in the development of incidence assays. However, several challenges still exist in calculations of incidence estimates due to adjustments, which are required for these assays to consider the false recent rate and those who are on ART. Furthermore, challenges also exist in implementation of incidence assays in the field, which include the large number of samples needed for incidence or FRR studies, a lack of guidance, and a depleting specimen repository.

Most of the surveys do not take into account the design effect of non-simple random samples, which should be considered in sample size calculations. More guidance is required for the field and the use of the assays and how these effects could affect incidence calculations.

Many countries are conducting HIV incidence testing on specimens from small surveys. Strong justification for the inclusion of incidence assays should be included in the protocol for smaller at-risk populations due to the sample size required for incidence estimation. Although guidance is required for implementation of incidence assays, issues related to the lack of conclusions on how to appropriately calculate or interpret incidence rates based on these assays should be considered in making recommendations. Incidence estimates in the field should be interpreted in context and consideration should be given to characterizing recent infections in populations when sample sizes are not adequate for accurate incidence estimation.

The second half of this session was an overview of the progress and challenges in estimating incidence in the field and the application of incidence assays.
Main Conclusions and Recommendations at the end of the session

- Regional workshops should be convened to introduce new concepts and detail the implications of using incidence assays
- Guidelines should be created for countries despite limitations in methodology
- Clear guidance is necessary not only for implementation of incidence studies in the field but also in the interpretation of results.
- Progress should be directed towards elongating the MDR and minimizing FRR. This in turn will result in smaller sample sizes being required to conduct incidence studies.

5.2 Session 2: Statistical Challenges for Incidence Estimation and Report from Statistical Consultation Meeting

In this session a summary of recommendations made and challenges discussed during the statistical meeting (18-19 August) were reported.

A new equation was proposed for estimating incidence that defines the duration of recency as the time to a fixed time (T) that an individual spends in the recency state.

i. This allows/requires different specimen sets for estimating mean duration of recency and FRR

ii. Simulations will be conducted to refine panel specifications, but collecting any such panels will be challenging due to the increasing number of people receiving treatment soon after seroconversion.

iii. Other statistical challenges include the use of multiple-assay algorithms, the calculation of incidence from complex surveys, and the need to avoid a fourth “exclusion” outcome when classifying assay/algorithm results

Main Conclusions and Recommendations

- With the new incidence equation, new FRR studies will need to be conducted, but specimen sets for these studies should be easier to acquire since they will not need to include recent seroconverters.

- The determination of the fixed time T needs to be discussed further in relation to different types of studies, scenarios, and assays.

5.3 Session 3 Session 3a and 3b: Update on specimen repositories and new cross-testing initiatives

CDC and the Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA), funded by BMGF, are working to create specimen repositories that will provide standardized sample sets for
validating new incidence assays. Both are collecting longitudinal, cross-sectional, known long-term infections, and challenge panels with sufficient volumes of specimens to be used for this purpose. These panels are in high demand from the assay developers; however the cost to collect and maintain such sample sets is proving to be problematic for the repository hosts.

CDC has created an internal Incidence Core Team (ICT) with the goals of sharing information within the CDC, developing standardized protocols, and providing guidance for laboratory based incidence estimation. CDC has four incidence assays (BED, LAg-avidity, rIDR-M AI, and Bio-Rad avidity) ready for a cross-comparison, and has begun sharing specimens for this purpose across CDC’s Division of HIV/AIDS Prevention (DHAP) and the Division of Global HIV/AIDS (DGHA), also known as the Global AIDS Program (GAP).

CDC is in the process of creating an internal specimen repository of longitudinal, known long-term, cross-sectional, and challenge panels specimens for HIV incidence assay validation. Thus far, they have compiled inventories of previously collected field specimens, collected panels from two known recently infected cohorts, the Seroincidence Panel Project (SIPP) and Thai MSM, and have planned to collect a panel from 100 seroconverters in Kenya.

A field evaluation in Swaziland (SHIMS) is currently underway to estimate HIV incidence rates using laboratory based incidence tests and to compare estimated incidence rates from the BED, LAg, and Bio-Rad avidity assays to directly observed rates.

The high cost ($2 million for the SIPP study) of collecting seroconverter panels will make it more difficult to obtain new panels or to expand the project.

The CEPHIA project, led by the Health Protection Agency (HPA) has four main objectives:
1) Create a specimen repository composed of large panels of HIV+ specimens for RITA assay/algorithm assessments
2) Fully evaluate 10 candidate assays for ability to correctly identify recent HIV infection
3) Derive and deploy improved methodology to estimate RITA mean duration of recency and false-recent rates, and further to determine algorithms that may be constructed from available components/tests
4) Build collaborations and consensus around characterization and deployment of RITAs in the field

Funded by BMGF for three years, CEPHIA is in the process of creating a specimen repository which will be available to developers to evaluate the performance of HIV incidence assays. They have created a developmental specimen set of 50 low challenge specimens which is available upon request, as well as a qualifying panel with 250 specimens that is available at CEPHIA’s discretion. CEPHIA is also collecting challenge panels with known confounders of incidence assays.

Some of the issues discussed during this session highlight that there are a large number of specimens at CDC and NIH that could be used to validate new assays; however IRB regulations make it difficult to share these specimens with other groups. Although this task is not impossible, gaining access to current cohort specimens collected for incidence testing requires a lot of paperwork and generally takes 6-9 months.
On the other hand, several seroconversion cohorts are facing de-funding. Maryland is in the process of obtaining 63 thousand specimens and their respective databases that the NIH can no longer maintain, which could be a possible resource for new assay validation. The current criteria for obtaining the qualifying set of specimens is fluid and at the board’s discretion.

Another issue raised was that replenishing repositories in the future may be difficult due to earlier initiation of ART.

**Main Conclusions and Recommendations at the end of the Session 3A**

- CDC should try to share the SIPP specimens with CEPHIA.
- SIPP specimens have information on CD4 counts and viral load and CDC is trying to collect the same information for the Kenya panel.
- We need to maintain funding for current projects and/or sift through IRB protocols to ensure there are sufficient specimen sets for incidence assay validation.

**5.3 Session 3b: Incidence Assays under Evaluation**

This session introduced several promising new assays for laboratory-based HIV incidence estimation, which utilize antibody avidity or reduced sensitivity to determine recent HIV infection and include both modified commercial assays and assays developed in-house. All of the assays have been evaluated in-house, but the assays need to be further evaluated using standardized criteria and a standard specimen set in order to directly compare assays and fully understand their performance.

CDC described two new assays for determining recent HIV infection: a two-well avidity index assay and a single-well limiting antigen (LAG) avidity assay. So far the results are promising— the mean recency periods at a cut-off of 1.0 were similar across subtypes A/D, B, and C for the LAG assay, and the FRRs for long-term infections and AIDS patients in Vietnam were 2.3% and 0.2%, respectively. Technology transfer has been completed and kits are currently being created. Once manufactured kits are available, the assays will be validated in other labs, including UCSF, HPA, and JHU.

Another new assay developed by CDC is a modified 3rd generation Bio-Rad EIA that uses standard EIA equipment to determine recent HIV infections from cross-sectional studies. The mean recency period for the assay has been determined at several avidity cut-offs, which were statistically similar between B and non-B subtypes for most avidity cut-offs. The FRR from long-term infections was below 5% for subtype B, C, A, and D specimens from 4 different countries; however was increased in specimens from Kenya and Uganda, which were primarily subtypes A and D. The history of ARV use was shown to affect the accuracy of the assay. The Bio-Rad EIA covers multiple antigens/epitopes, unlike the BED and rIDR-M assays that focuses only on gp41.

Another two incidence assays have been developed based on reduced sensitivity or antibody avidity using an Ortho Clinical Diagnostics Vitros clinical system. The less sensitive (LS) assay had a mean recency duration of 146.0 days at a S/C ratio cut-off of 20 and the avidity assay had a mean recency
duration of 169.7 days at an avidity cutoff of 0.6. Both assays combined (a Vitros LS and avidity combination) had a mean recency duration of 169 days and a false recent rate of 7%. An in-house validation and in-house evaluation of these assays have been completed. A field evaluation is in the planning stage.

The results for the Architect Avidity index assay were presented, as well. The 4th generation Architect HIV Ag/Ab, the 3rd generation AxSYM HIV ½ gO, and the 3rd generation Vitros Eci HIV 1-2 HIV tests were evaluated for their ability to detect recent infections using antibody avidity and a pre-defined window period of ≤ 6 months. The FRR of the Architect HIV Ag/Ab, the AxSYM HIV ½ gO (cut-off 0.8), Vitros Eci HIV 1-2 (cut-off 0.5) were 6.6%, 2.6%, and 20.9% when samples from patients on ART were excluded from the analysis. Precision analysis was performed for the Architect HIV Ag/Ab and a coefficient of variation was determined to be 5.1% +/- 2.2%. The cost with Architect is about $9 per sample (two wells).

Some of the issues raised during the discussion following this session were:

- The developers need to run the assays on a standardized sample set to be able to compare mean recency windows and FRRs among the assays.
- The working group needs to develop standardized criteria for assay developers to follow, including a final pass/fail step.
- 4th generation commercial assays can be modified to avoid complicating results due to detection of p24 antigen-- Bio-Rad and Vitros have indicated that the "detection" reagent for p24 could be omitted from the tests and thus likely eliminate this issue without compromising the antibody portion of the tests.
- The new assays need to be evaluated in populations with a known incidence.
- For assay evaluation, we should rely on the specimen repository sets instead of large, costly field evaluations.
- It is beneficial for incidence assays to cover multiple epitopes.
- It is unclear to both the developers and the users as to when it will be acceptable to replace the BED with a new assay for incidence estimations in the field.
- The incidence assays currently being developed perform poorly on specimens from patients on ARVs--all have a high FRR on specimens from treated patients. Developers need to address this issue as the number and proportion of patients on treatment is expected to increase.
- Although concerns were raised that CDC may be pushing the LAg assay out prematurely, many validation studies, ongoing and planned for coming months, will be conducted before the assay is rolled out for routine use. Studies include field validation of incidence estimates and determination of FRRs and mean duration of recency.

Main Conclusions and Recommendations for Session 3B

- The working group needs to develop a set of standardized criteria that must be met for an assay to be deemed fit for incidence estimation.
- A standardized set of specimens is needed for assay development and head-to-head comparisons of assays.
- We still need additional data for non-B subtypes.
- A precision study should be conducted for every new method.
- A pre-defined mean duration of recency can be used, as is the case in several other avidity assays.
Developers need to address the issue of high misclassification of specimens from patients on ART.

Session 4: August 23, 2011

5.4 Session 4a: Tests for recent infection on the horizon

Three assays for the determination of recent infection that are currently under development were presented: Luminex, Rapid I-P, and High Resolution Melting (HRM). Luminex and Rapid I-P are based on antibody detection and the HRM diversity assay is nucleic acid based. Luminex and the HRM diversity assays are lab-based assays, while the Rapid I-P could be performed in the field. All are at the stage of in-house evaluation and validation. Detailed methodology, sample type and volume (all assays currently use serum or plasma), required equipment, cost, results on recent vs. non-recent and seroconversion/longitudinal samples were presented. Another presentation in this session focused on individual level disease staging, the public health implications and consequences of patients receiving acute infection status, and the ability of current assays to detect acute infection.

5.4.1 Development of a Bead-Based Multiplex Assay for the Determination of Recent HIV-1 Infection

The Luminex assay uses microspheres to capture and detect antibody to multiple HIV antigens in a single well, using 1 micro liter of serum or plasma per well. The amount of antibody and antibody avidity can be measured if 2 wells are used and up to 7 analytes/measures can be used to differentiate recent from non-recent samples. This assay can also be used for measurement of different subclasses of antibody with minimal modifications to the protocol.

FRR calculations with a mean recency period around 180 days were shown for individual analytes. The percent false recent using a combination of five analytes with the best separation in antibody reactivity between recent and long-term (LT) specimens was 0.2 among LT specimens from patients without AIDS and 0.1 among LT specimens from those with AIDS. The avidity measures appear to be more robust and less affected by AIDS/ART. However multiple analyte combinations/algorithms may improve potential misclassification due to immune variation and factors that affect the immune response. IgG3 antibody to p24 and p66 also appear to be promising indicators of acute infection. The cost in-house cost is about $11.44 per sample (2 wells).

5.4.2 Rapid detection of HIV prevalence and incidence using a single test device.

The Rapid I-P (RIP) is a simple rapid test device with characteristics of current rapid tests and uses only 1 µl of serum or plasma. This test simultaneously detects HIV-1 incident and prevalent infection. It is a simple, rapid device; robust, portable, and field applicable.

So far results have been obtained from testing seroconversion panels, low titer specimens, cross-sectional specimens from multiple subtypes, and long-term infected. The long-term status was confirmed in 98.5% of the specimens (652/662). Seven specimens appeared to be recent by days since last negative and by both BED and RIP; one patient had six false recent results; four with CD4 < 50, at days >3000 and four patients with one false recent, three with CD4 <30.
The RIP assay appears to be unaffected by HIV-2 prevalence, negative specimens, subtype O or the presence of multiple subtypes.

Improvements to the assay now under evaluation include: a change from the recombinant gp41 antigen to a multi-antigenic peptide (MAP) to eliminate cross-reactivity with HIV-2 and use of a portable reader to eliminate subjective interpretation. The next steps include kit preparation, generation of large lots for stability studies, additional data sets to determine window period and FRR, and field studies for acceptability and reproducibility. Participants raised issues about the need to compare results with EIA tests and to optimize the incident line to match BED-ELISA results. In spite of the promising results it is too early to assess how the RIP would be applied. The use of whole blood will be evaluated in the future.

5.4.3: Analysis of HIV diversity using a high resolution melting (HRM) assay

HIV diversity changes with time in individuals with HIV infection. In general, there is an increase in diversity during the asymptomatic phase of infection and a plateau in diversity in late infection. These generalizations are based on a relatively small number of studies of HIV diversity that analyzed sequences of individual viral variants. HIV diversity is being explored as a biomarker for HIV incidence determination. Use of HIV diversity for incidence determination would be facilitated by use of a rapid assay that does not require sequencing

The HRM diversity assay is scalable and does not require sequencing. The assay provides a numeric score (HRM score) that is associated with sequence-based diversity measures. HRM diversity data from individuals with acute, recent, and non-recent HIV infection was presented. This included data from six different genomic regions. Non-recent infection samples included a high proportion of samples from individuals who had low CD4 counts at the time of sampling or had been exposed to ARV therapy; these samples are often misclassified as recent using serologic HIV incidence assays. HRM scores were significantly lower in recent compared to non-recent HIV infection. Some adults with non-recent HIV infection had low viral diversity in one or more regions of the viral genome but did not typically have low HRM scores in all regions analyzed, distinguishing them from adults with recent infection. Furthermore, HRM scores in three regions of the genome were independently associated with non-recent infection, indicating that multi-region analysis HRM diversity may provide more robust discrimination between recent and non-recent infection than analysis of a single genomic regions.

On-going studies are evaluating use of the HRM diversity assay as one part of a multi-assay algorithm (MAA), in which samples are first screened for recent infection using serological assays. This work includes an evaluation of the impact of HIV super infection and viral suppression on HRM diversity scores.

5.4.4 The potential for use of RITAs in individual level disease staging

Physicians would like to have the information about whether individual, newly diagnosed patients are acutely infected, as this information has both clinical and public health implications. Detection of acute infection is both a unique clinical situation and a public health emergency. The approach to managing patients with acute infection is different than for chronic infections with regards to the immediate emphasis placed on partner testing and avoiding HIV transmission; the urgency of genotyping, and
consideration of early ART initiation (which is felt by many but not all physicians to be indicated in acute infection). From the public health perspective, contact tracing for partners can be prioritized as a prevention activity in cases of acute HIV infection.

Moreover, it was emphasized in this session and in field reports (see section 6.2.5, Activities in Kenya) that many key surveillance uses of RITA results are to classify individuals in surveillance datasets as having recent or non-recent HIV infection—and using analysis of factors associated with recent HIV infection as a way of tracking the “leading edge” of an epidemic. To classify individuals’ recency status for this type of application, RITA results do not need to be as highly accurate as they would preferably be for reporting to physicians.

This WHO Working Group’s previous market analysis suggested that demand for incidence tests will be much greater if they can have a dual purpose—i.e., if they could be used for individual level classification as well as incidence estimation. To be most appealing for use by testing programs, the assay could have a role in diagnostic testing algorithms. Examples of incidence tests also used in clinical algorithms could include rapid tests and multiplex assays such as the Western blot. In addition to the above rapid I-P test, new data were presented on the potential performance of the Chembio DPP HIV Incidence test—a multiplex assay similar to the DPP HIV1/2 confirmatory assay used clinically every day in Brazil. In addition, results were shown from a recent study demonstrating performance of commercial western blots for classifying individual patients as having infection of less than 90 days duration: criteria based on the number of positive bands performed equivalently to various detuned assays and the BED for individual disease classification. In summary dual purposing of assays should be highly considered about their utility. With a much bigger market, companies may be interested in investing in this field.

In some countries, such as the UK and Italy, physicians are routinely given the avidity results, and research is forthcoming regarding surveillance for any possible adverse consequences of individual level reporting for physicians, patients or their partners. In the meantime, there was agreement that information is useful for clinical management, partner notification treatment, etc. and having a dual test will be beneficial.

6.1 Session 4b: Update on new results from TRI field evaluation

Results from field evaluations in several countries were presented in the morning session. FRRs were determined in Vietnam using BED and LAg-Avidity EIA. In Ghana, 3 assays are being evaluated—BED, LAg-avidity and rIDR-Avidity. Only BED testing and FRR calculations have been completed. A sequential testing algorithm will be evaluated. Regional determinations of FRR are critical.

6.1.1: Determination of false recent rates for the measurement of HIV Incidence in Vietnam

A cross-sectional study using BED-CEIA and the LAg-Avidity EIA was conducted in Vietnam with the following objectives: (1) to estimate the FRR among patients with long-term infection by age, gender, duration of infection and CD4 level; and (2) to compare the FRRs of both assays.
The study team collected blood specimens from ARV-naïve persons documented to have been infected with HIV for >1 year and who attended care services between April 2009 to December 2010 in clinics located in the Northern and Southern regions; chart reviews were conducted to obtain demographic and HIV clinical status data.

Significant differences in FRRs were found between Northern and Southern regions with both assays and further investigation is needed to understand these differences. The LAg-avidity EIA FRR was lower than the BED FRR (2.33% vs. 3.58%). The BED FRR results were 5.86% for the North vs. 1.03% for the South; and for LAg-Avidity FRR results were 3.91% for the North vs. 0.57% for the South.

Genotype testing was conducted to determine whether subtype could explain the FRR differences between the two regions, but all the specimens were the same HIV-1 subtype (CRF01_AE). Western blot was conducted to further understand the reasons for false-recent classification—15% of all false-recent infections had Western blot results indicative of recent infection, and nearly all were from the North.

Duration of infection was determined using documented test dates but was not precise because some specimens were obtained 12 months apart.

**6.1.2: Update from a false-recent rate study in Ghana**

The objective of the study in Ghana was to estimate the FRR among patients with long-term infection by age, gender, length of infection and CD4 level using three assays, BED, rIDR-avidity, LAg avidity, and compare results of the individual assays with sequential incidence testing algorithms.

Blood specimens were collected at eight ART clinics located in six regions from ARV-naïve persons documented (by HIV diagnosis date) to have been infected for longer than one year. Medical record abstractions were conducted to collect data on age, gender, exposure category, length of infection, and most recent CD4 count.

Preliminary results showed that BED testing has an FRR of 6.40%. Other testing is pending and will be performed at CDC.

Some critical issues raised during the Ghana field study were that recruiting patients who come to a clinic seeking care could potentially bias the sample towards those with advanced disease. In this study there were no differences in CD4 counts. The duration of infection is difficult to verify without good documentation. In addition, it is difficult to determine local FRR in regions with very low incidence, and incidence calculations using current assays will not be accurate without this information.

Based on results from Vietnam, regional difference in FRRs should be considered in other countries when evaluating incidence assays. Whether persons in any clinic cohort should be considered nationally representative was discussed. Potential issues of regional patterns of ART usage and access to ART should be considered. For example, ART uptake in Vietnam was higher in the South compared with the Northern region.

The afternoon session featured results from field evaluations and incidence studies from the following countries: Vietnam, Ethiopia, South Africa, Mozambique, East Africa, Kenya, and Central America. A new
multi-assay algorithm for cross-sectional incidence testing was presented. In addition, a summary of the incidence surveillance and estimation methods and recent trends in incidence in the US was presented.

6.1.3: BED-CEIA Assay: HIV Incidence Estimation and FRR Results from Six International Sites

As part of a USAID-funded initiative to increase developing country capacity to conduct HIV prevention and clinical research, especially for topical microbicide evaluations, HIV-1 incidence studies were funded in six international sites (HCMC, Vietnam; Rustenburg, RSA; Bloemfontein, RSA; Addis Ababa, Ethiopia; Chokwe, Mozambique; Beira, Mozambique) with the following objectives: to establish false recent rates (FRRs) for the BED-CEIA in each site, and to compare prospective measures of incidence with adjusted cross-sectional estimates. The studies have been completed in Vietnam and South Africa (RSA), but are still ongoing in Ethiopia and Mozambique (MZ).

In each site, a cross-sectional survey was conducted in populations of sexually-active women at high risk for acquiring HIV. The survey consisted of interviews and testing for HIV and pregnancy, and testing HIV-positive samples with the BED assay to estimate incidence. Women initially found to be HIV-negative in the cross-sectional survey were then enrolled in a prospective follow-up phase and followed for 6 to 24 months to obtain a direct measurement of incidence. Specimens from men and women, aged 18-35 years, with established HIV infection for longer than 12 months, as documented in medical records and no history of ARV therapy were tested with the BED assay to determine BED FRRs.

HIV prevalence varied widely in these settings: 7.8% in Vietnam (excluding opiate-positive participants, but 14.1% with their inclusion); 10.9% in Ethiopia; 21.2% in Bloemfontein, RSA; 23.5% in Rustenburg, RSA; 31.3% in Chokwe, MZ; and 33.5% in Beira, MZ.

The overall BED FRRs (among both men and women) were 4.3% in Addis Ababa, Ethiopia (95% CI: 2.4-5.9, N=486), 3.4% in Chokwe, MZ (CI: 1.5-6.6, N=235), 1.6% in Beira, MZ (CI: 0.3-4.6, N=189), and 1.7% in Ho Chi Minh City, Vietnam (CI: 0.7-3.6, N=403).

Corrected cross-sectional incidence estimates were calculated using local FRRs (except for South Africa estimates, which used the FRR from CDC guidelines for use of BED) and a 197-day mean recency period. Even after adjustment using various methods, the cross-sectional incidence estimates were higher than the incidence estimates from the prospective follow-up phase.

A comparison of the adjusted (Hargrove method) cross-sectional incidence rates with the prospective incidence measure found good agreement between these two respective measures of the rates for Vietnam (0.5% [95% CI: 0.0 - 0.9] vs. 0.0% [CI: 0.0 - 4.3]), Bloemfontein, RSA (9.2% [CI: 6.7 – 11.6] vs. 5.5 [CI: 2.5 – 10.4], and Beira, MZ (8.6% [CI: 4.8 – 12.2] vs. 6.6% [CI: 3.2 – 12.2]). For Rustenburg, RSA, lack of robust sample size and a local FRR estimate (due to issues with medical record access for confirmation of HIV testing) limited the comparison between the cross-sectional and prospective incidence measures (14.1% [CI: 9.5 – 19.3] vs. 3.0% [CI: 0.4 – 10.8]). Similarly, there are little prospective data available yet for Chokwe, MZ, so the comparison between these two measures for this site is not compelling (8.7% [CI: 5.3 – 12.2] vs. 4.5 [CI: 0.1 – 25.1]).

Despite limitations in the study with the limited medical record access for BED FRRs at the RSA sites, limited sample sizes, and having only preliminary data from some of the sites, the study shows the utility
of calculating local BED FRRs. However, sample sizes must be sufficient for calculating more precise estimates.

It was suggested that a multi-test RITA is likely to be more accurate than a single assay for estimating incidence.

Concerns were raised about the possible impact of various factors on the calculation of local FRRs and the incidence estimates, including study inclusion criteria, unknown duration of HIV infection, and the reliance of self-reported information on ARV use.

There was discussion regarding increasing prevalence with age, as is seen in the epidemic in South Africa, and whether there is a need to obtain age-stratified FRRs in populations with age-related variability in prevalence. For this study, small sample sizes would make it difficult to get FRRs for more specific strata.

6.1.4: Frequency of Misclassification by BED-CEIA and BioRad Avidity Assay across different Geographic Regions in Africa

Researchers at Johns Hopkins University have used the BED-CEIA and BioRad Avidity assay to test a variety of cohorts across numerous geographic regions, with samples from the major subtypes (B, A, C, and D) and from different risk groups (MSM, injection drug users, heterosexuals). These cohorts have contributed 9,989 samples from 2,554 known seroconverters, and include 799 specimens from persons known to be infected < 1 year, and 5,859 known infected ≥ 2 years.

Using the Avidity and BED assays in a dual-assay algorithm with the current cutoffs for defining recency (BED: OD-n < 0.8; Avidity: AI < 40%) minimizes the potential for the BED assay to include individuals that are falsely classified as having recent HIV infections, but may miss including individuals that are truly recently infected. Thus, a theoretical framework for a new multi-assay algorithm (MAA) for cross-sectional incidence testing was developed.

The proposed MAA uses the BED and Avidity assays, but with higher cutoff values used for defining recency (BED: OD-n < 1.0; Avidity: AI < 80%) and includes the use of CD4 and viral load data to improve the classification of recency, including only data with CD4 cell counts > 50 (to exclude really long-term infections) and viral loads > 400 (to increase specificity).

For clade B, Kaplan-Meier survival analysis produces a 50% survival estimate for the MAA of 162.5 days (95% CI: 142 – 180.5).

In an analysis examining the risk factors associated with misclassification by the BED assay in 603 chronically infected (2-8 years post-infection) MSM, the following factors were significantly associated with misclassification: low viral load (<400), low CD4 (<200), HAART (2+ years), and non-white race. The odds of misclassification among blacks/African Americans were twice that among whites.

The performance of the MAA in a clade B epidemic was evaluated in the HIVNET-001 cohort to assess the window period, and in the ALIVE, MACS, and Moore Clinic cohorts to assess specificity. The window period for the MAA was 163 days (95% CI: 142 – 181) compared to 175 days (153 – ?) for BED alone.
(using a cutoff of OD-n < 0.8), 135 days (CI: 115 – 161) for Avidity alone (Al < 40%), and 176 days (CI: 112 – 198) for BED and Avidity combined (BED OD-n < 1.0; Al < 80%). Specificity for the MAA was 99.5%, compared with 84.3% for BED alone, 98.9% for Avidity alone, and 97.4% for BED and Avidity combined. Specimens from the Partners Study were used to evaluate misclassification in non-B clades. The percent of specimens misclassified by the MAA was 1.4%, compared with 7.6% for the BED assay (OD-n < 0.8) and 3.5% for the Avidity assay (Al < 40%).

Factors associated with misclassification were also evaluated using specimens from the Partners Study. While no significant risk factors associated with misclassification were found with the MAA, there were significant risk factors associated with misclassification for the BED assay (viral load < 400, CD4 > 500, and being from Uganda or Kenya) and for the Avidity assay (being from Uganda or Kenya). For the BED assay, the odds of misclassification for specimens from Uganda was twice that of specimens from South Africa; for the Avidity assay, the odds of misclassification was 8 times higher for Ugandan specimens compared with South African specimens. It was speculated that the higher misclassification in Uganda might be due to differences in the host virus (e.g., subtype differences).

The next steps in evaluating the MAA include confirming its generalizability, conducting further testing with non-B cohorts and other populations, finding an alternative to CD4 data (due to the difficulty of obtaining CD4 results at the time of blood collection), optimizing the window period vs. the specificity, and evaluating other incidence assays (Vitros-LS, Luminex, and LAg).

The main issues raised were the concerns about a high field cost for the algorithm. It was noted that in some areas CD4 and viral load data are frequently collected on a regular basis.

There was discussion regarding the trade-off between the benefit of having a longer window but sacrificing specificity. From the laboratory perspective, it is best to have the smallest amount of misclassification as possible.

6.1.5: Kenya Incidence Activities

CDC DHAP and DGHA are collaborating to conduct a seroconverter cohort study, to perform field validation of CDC incidence assays in existing cohorts, and to analyze data from completed surveys. The preliminary results were presented from the cross-assay analysis in the 2007 Kenya AIDS Indicator Survey (KAIS).

The KAIS was a national household survey with a two-stage cluster sample design. The sample size was 10,025 households with 17,940 participants. 15,583 (87%) of the participants provided blood for testing. Two tests for recent infection were applied: the BED-CEIA and the Lag-EIA. The assay-derived incidence was compared with mathematically modeled incidence (both EPP/Spectrum and survey-derived). The mean duration of recency used for incidence estimation was 197 days (95% CI 173 – 220) for the BED and 141 days (95% CI: 119 – 160) for the LAg. The FRRs used to adjust the assay-derived incidence estimates were 15.4% for the BED and 3.8% for the LAg.

FRRs were estimated from known chronic infections in KAIS. Although the FRRs for the LAg were lower than the BED, they were still moderate. The FRR among participants on ARV was lower than expected for the LAg. Among HIV-infected persons currently on ARV, the percent misclassified was 26.7% for the
BED, and 4% for the LAg. Among ART-naive HIV-infected persons, the percent misclassified as recent was 15.4% for the BED and 3.8% for the LAg.

National HIV incidence estimates derived using four different methods were compared. The results were as follows:

- EPP/Spectrum model: 0.72% (plausibility bounds 0.7 - 0.74)
- Survey-derived (Hallett 2008): 0.7% (95% CI: 0.3 – 1.1)
- BED Assay-derived in KAIS (unadjusted): 2.1% (CI: 1.7 – 2.5)
- BED Assay-derived in KAIS (adjusted): 0.5% (CI: 0 – 1.8)
- LAg Assay-derived in KAIS (unadjusted): 1.1% (CI: 0.8 – 1.4)
- LAg Assay-derived in KAIS (adjusted): 0.5% (CI: 0 – 1.2)

The unadjusted incidence estimate for the LAg fell within the plausible range of the Hallett model. The distribution of recent infection varied by assay type, particularly for the following characteristics: condom use, HSV-2 infection, circumcision, and location of residence (largest proportion of recent infections observed with BED was in Nyanza whereas the largest proportions observed with the Lag were in the Rift Valley). “The unadjusted incidence estimate for the LAg fell within the plausible range of incidence from the mathematical models. The distribution of recent infection for select variables varied by assay type (e.g. more recent infection in Nyanza observed with the BED whereas more recent infection in the Rift Valley observed with the LAg).

Some factors were found by both assays to be associated with recent infection, although other factors were found by one assay to be associated with recent infection, but not the other. For example, among women, factors identified by the BED to be associated with recent infection were HSV-2 infection, younger age, and residence in the Nyanza province; factors identified by the LAg were HSV-2 infection, younger age, low educational attainment, and currently being married. Among men, factors identified by the BED to be associated with recent infection were older age, multiple partners, genital ulcer, and lack of circumcision; factors identified by the LAg were multiple partners, genital ulcer, and residence in Rift Valley.

Data are preliminary; these are the first results from the LAg field evaluation, and additional validation studies in different settings around the world are needed.

The validation of these incidence estimates was limited to comparisons against mathematical models. The FRRs were based on a small sample set within the national sample, and although they may be representative, they may not be valid estimates.

The next steps include applying the BioRad Avidity assay to the same sample sets, and conducting incidence testing using multiple candidate assays for the second round of KAIS.

There was a question regarding the extent of the overlap between specimens identified as recent by the BED and specimens identified as recent by the LAg—all specimens identified as recent by the LAg were also identified as recent by the BED.
It was noted that avidity testing using the same epitope as the BED won’t be as robust as using a test based on a different epitope, which is shown to work well independently; it may be better to use orthogonal assays.

There was a question regarding whether the data for the risk factor analysis were adjusted for false recent infections. The risk factor analysis used unadjusted data, so results may be affected by potential misclassification.

6.1.6: Using the available tools to determine incidence in Central America

Central America has concentrated epidemics. In 2010, HIV prevalence among the general population was less than 1% for most countries, except for Belize, which had a prevalence of 2.1%.

Integrated behavioral and biological surveys were conducted to determine behavioral risks for HIV and STI and to determine HIV prevalence and incidence among MSM, Female Sex Workers (FSW), and Garifunas residing in Honduras, El Salvador, and Nicaragua.

Participants were recruited among MSM and FSW by respondent-driven sampling, except for FSW in Nicaragua, who were recruited by time-location sampling. Garifunas were recruited through stratified household sampling. A behavioral questionnaire was administered through audio-assisted self-interviews.

The FRR for El Salvador and Honduras was estimated using data from 800 known HIV-positives from El Salvador; the FRR for Nicaragua was estimated using 200 known positives from Nicaragua. Positive samples were tested with the BED assay. For El Salvador, the BED FRR among all specimens from persons with chronic HIV infection was 19.8%, 22.6% among those that from ARV-treated persons, and 10.7% (95% CI: 8.9 – 11.3) among those that were ARV-naïve. The FRR of 10.7% was used for calculating HIV incidence estimates for both El Salvador and Honduras.

The El Salvador BED FRR increased with increasing time on ART (based on self-report). The El Salvador BED FRR also varied by CD4 cell count, with a near 0% FRR for CD4 cell count <350, and 12% for CD4 cell count > 350.

BED HIV-1 incidence estimates for 2008 were calculated for MSM and FSW in El Salvador, using the FRR-adjusted formula for incidence and using a mean duration of recency of 155 days for the BED assay.
- Among MSM, annualized BED HIV-1 incidence was 7% (95% CI: 4 – 10), based on 21 (27%) recent specimens out of 79 available HIV-positive specimens. Incidence was higher among younger MSM (10% [CI: 5 – 14]) compared with MSM aged 25-34 years (3% [CI: 0 –5]), and MSM aged 35+ (0%).
- Among FSW, annualized incidence was 0% (CI: 0 – 2), based on 1 (3%) recent specimen out of 37 available HIV-positive specimens.

Comparison of data on HIV among MSM in El Salvador from 2003 and 2008 indicate that while HIV prevalence decreased (from 15% in 2003 to 11% in 2008), HIV incidence increased (from 0% in 2003 to 7% in 2008).
BED HIV-1 incidence estimates were also calculated for subgroup populations in Honduras as well, where nearly all (99%) of the circulating subtypes were subtype B.

- Among Garifunas, BED HIV-1 incidence was 0.4% (95% CI: 0 – 1), based on 5 (16%) recent specimens out of 32 available HIV-positive specimens.
- Among MSM, BED HIV-1 incidence was 1% (CI: 0.2 – 2), based on 6 (19%) recent specimens out of 31 available HIV-positive specimens.
- Among FSW, BED HIV-1 incidence was 0%; there were no recent specimens out of the 19 available HIV-positive specimens.

The Honduras BED incidence estimates for 2006 were compared with the 2007 modeled HIV incidence estimates using the Modes of Transmission (MOT) model. BED and modeled incidence estimates were very similar for MSM (1% modeled HIV incidence) and Garifunas (0.3% modeled incidence). Modeled incidence was 0.2% FSW.

In Nicaragua, the BED FRR was 9.7% (95% CI: 4.3 – 15.1). The BED HIV-1 incidence estimates for Nicaragua for 2010 were as follows:

- Among MSM, BED HIV-1 incidence was 3% (CI: 0 – 6), based on 11 (22%) recent specimens out of 51 available HIV-positive specimens.
- Among FSW, BED HIV-1 incidence was 1% (CI: 0 – 2), based on 4 (25%) recent specimens out of 16 available HIV-positive specimens.

The challenges for the BED approach to estimating HIV incidence in Central America are the small sample sizes and the high FRRs. As it is not possible to reach the sample sizes needed within each country, the presented suggested that results might be pooled with neighboring countries with similar epidemic patterns and HIV subtypes to obtain regional estimates. As incidence is highly sensitive to the FRR, estimating this parameter with good certainty is critical in Central America.

Despite issues with sample size for estimating HIV incidence, BED has been an important tool for HIV surveillance and advocacy in Central America. But it was acknowledged that because these results were not very accurate, better, more robust, sensitive and specific tests for incidence are needed for concentrated epidemics.

There was an observation that this presentation was a good example of the benefit of doing descriptive analyses of recent infections, without calculating incidence rates. It may be enough to capture the majority of recent infections, and to accept some level misclassification.

**6.1.7: HIV Incidence in United States, 2006-2009**

HIV incidence surveillance in the US is integrated with core surveillance activities. All HIV surveillance is state-mandated and governed by state regulations, and is done differently in different states. There are 25 states funded to obtain testing history and ARV treatment information (TTH) and Serologic Testing Algorithm for Recent HIV Seroconversion (STARHS) results. Diagnostic, Western blot positive specimens are obtained from state public health laboratories and from private/commercial laboratories, and are sent to the CDC STARHS laboratory in NY for testing with the BED assay.
A stratified extrapolation approach is used to estimate HIV incidence in the US. In this approach, a weight is assigned to each diagnosis that is deemed recent, where the weight is the inverse probability that an individual would choose to test in the recency period. The weights for each of the individuals deemed recent are added together for the total incidence for the 18 areas with adequate data for estimation, and then the data are extrapolated to the areas that did not provide data through HIV incidence surveillance.

The probabilities used for the weights are calculated separately for repeat testers (individuals who have had a previous negative HIV test) and new testers (individuals whose first HIV test was positive). For repeat testers, the probability is determined by the testing frequency and the time from the last negative test to the first positive test, assuming the infection date is uniformly distributed in the inter-test interval. For new testers, the probability is estimated from a competing events model of the time to an HIV test vs. the time to AIDS diagnosis, assuming the HIV testing hazard is constant after HIV infection until AIDS diagnosis.

The first results from national HIV incidence surveillance using a Stratified Extrapolation Approach were published by Hall, et al. in 2008. The reported HIV incidence in 2006 was 56,000 (95% CI: 48,200 – 65,400). This report also included an extended back calculation estimate of incidence based on prevalence methods: this provided an annual incidence estimate for the most recent 3-4 year period from 2003-2006, and indicated the stability of incidence through the 2000’s.

HIV incidence estimates for 2006-2009 were published in PLoS ONE on August 3, 2011. This report provided the first multi-year estimates from HIV incidence surveillance using the stratified extrapolation approach, and included an update to the 2006 estimate as well as incidence estimates for 2007-2009. Only individuals aged 13 years and older at diagnosis were included, with a residence at diagnosis in the 16 states and 2 cities included in analyses. Individuals with AIDS diagnosed within 6 months of HIV diagnosis were classified as “long-term”, and the BED results for individuals on ART were set to missing.

CDC estimated that there were 48,600 (95% CI: 42,400—54,700) new HIV infections in the US in 2006, 56,000 (CI: 49,100—62,900) in 2007, 47,800 (CI: 41,800—53,800) in 2008 and 48,100 (CI: 42,200—54,000) in 2009.

The 2006-2009 HIV incidence estimates reflect refinements to the previous statistical model for HIV incidence estimation: it revises the way HIV diagnosis data are adjusted for reporting delay, provides a more accurate way of estimating the probability that an infection would be detected when it is recent, uses multiple imputation to determine transmission category data when that information is missing, and uses more recent data that allowed recalculation of the BED mean recency period, resulting in a revised period of time of 162 days (standard error 8.5 days).

For 2006-2009, incidence was stable, with a slight increase in 2007. Possible factors influencing the 2007 estimate were examined, including assessing the data to determine whether the increase could be due to a sudden increase in HIV testing or sudden changes in reporting practice, but there was no information to suggest that the data were impacted by these changes. There may have been an increase in incidence in 2007 but there is no clear explanation for that. The majority of new HIV infections were in MSM—in 2009, MSM accounted for 61% of new HIV infections. HIV incidence was essentially stable in all demographic groups except young African American MSM. Among MSM aged
13-29, HIV incidence among black/African American MSM increased significantly (48%) from 2006 through 2009 with a statistically significant 12.2% estimated annual percentage increase.

Concerns were raised about the assumptions that testing is independent of the reason for testing, because there is evidence of dependence between infection and testing. The US TTH data were initially examined to determine whether persons were choosing to get an HIV test because they thought they might have been exposed to HIV infection in the past six months. The problem was that a high proportion of individuals thought they might have been recently exposed to HIV. There was no difference found in the percent of BED recent among those that said they had recently been exposed to HIV and the percent BED recent among those that did not indicate recent exposure. It was also noted that among those with a short inter-test interval from the last negative to the first positive test, the observed proportion of recent infections was lower than what was expected based on the current survival function of the recency period. This indicates possible biases in the recall of dates or in the estimation of the recency period.

During the discussion following the session on country experience, many issues were raised. In summary, there was consensus that having a rapid incidence test would be helpful. Testing would increase, the representativeness of studies would increase, and costs would be reduced. Concerns were expressed regarding disclosing individual-level results, as any rapid incidence test would need to be a very good diagnostic tool in order to be used for giving individual results.

The use of dried blood spots (DBS) was also discussed. There are many advantages in using DBS, including the financial benefits from savings in freezer costs, shipping, and long-term storage. Countries with high humidity need to consider proper storage of DBS, including frequent replacement of desiccant. The possibility of oral fluid testing was brought up. This would be particularly helpful in Central America, where MSM prefer oral testing.

The availability of DBS, rapid, or oral tests is pertinent for the US, as the new expected algorithms for HIV diagnosis may limit the ability to obtain blood for BED testing. Using DBS would assist with increasing the number of specimens for the BED assay. It would also be helpful if the US could use a rapid test in the field. The US is considering the need to start thinking about how incidence might be estimated using all the relevant sources of available information.

Questions were raised regarding whether the US had calculated FRRs or performed sensitivity analyses using different levels of FRRs, whether there was any differential misclassification of recent infection by race, and whether there has been an update to the incidence estimates from back calculation. The US has not calculated FRRs, as other methods are used to minimize false recent misclassifications by setting the BED result to “long-term” for persons diagnosed with AIDS within 6 months of HIV diagnosis, and setting the BED result to missing for persons on ARVs. The focus is on looking at relative trends over time, rather than the specific estimated numbers of recent infections. Differential misclassification by race has not been considered in the US data. Analyses are currently being conducted for updated back calculation estimates for incidence—the current data so far indicate that incidence estimates are in the ballpark.

There was also a question regarding the number of specimens that were tested for the US estimates, and the number that were recent. Among individuals who had not had AIDS diagnosed within 6 months
of HIV diagnosis, the number with BED results by year (and percent of total diagnoses) was 6,096 (31%) for 2006, 7,615 (37%) for 2007, 8,863 (44%) for 2008 and 9,615 (50%) for 2009. Of those without a diagnosis of AIDS at or within six months of HIV diagnosis, after imputation, the percent classified recent by year was 31%, 33%, 31%, and 30%.

Discussion regarding the proposed multi-assay algorithm (MAA), included questions about how the proposed new assay cutoffs of OD-n < 1.0 for the BED and AI < 80% were selected, how the criteria for the MAA would be used to define recent infections, how to define the recency period using combined assays, and the method used to estimate the survival function for the recency period.

Recommendations were made about the need for guidance regarding: (1) factors for determining whether it is feasible to estimate incidence for a population (e.g., is there a population prevalence threshold or false recent rates that would make it inadvisable to estimate incidence); (2) acceptable minimal sample sizes (e.g., is a sample size of 50 acceptable for incidence estimation?); and (3) evaluation of country resources to determine which incidence estimation methods would be feasible and most beneficial.

Having seen the problems estimating FRR in the field there was a question about the use of proxy FRRs for countries that can’t do their own FRR studies. Because there are regional differences, it seems there is a clear need to do survey-specific FRRs. Having better tests that yield low enough FRRs would resolve this issue. It was suggested that it might be helpful to do sensitivity analyses, using a Monte Carlo approach to determine the impact of uncertainty around the FRR on incidence.

**Sessions 5-6: August 24, 2011**

7. Session 5: Update on development of WHO guidance documents for HIV incidence assays

In 2009 documents on the validation of HIV incidence assays and estimation of HIV incidence were drafted. The new guidelines on how to use HIV incidence assays to estimate HIV incidence at the population level was finalized and published in 2010. These guidelines were based on some of the material developed in the original 2009 document. In the last working group meeting held in Geneva in 2010, it was recommended that the validation document should be another component, separate from the guidelines.

The next steps in the critical path were presented. During 2011 there was an agreement on high level of development approach, but not on the definition of candidate assay evaluation criteria. A new validated assay should be available, however, for use by 2014.

Document 1 titled “Guidance for the development, validation and evaluation of assays” used to estimate HIV incidence” was introduced to the audience. It was proposed that the document include: introduction, key definitions and terminology, concept, product planning (profile and specifications),
specimens sets and panels, assay development, independent assay evaluation in reference labs, pilot testing in the field using prototype kits, scale-up commercialization, algorithms, and cost considerations.

Document 2 titled “Guidance for the field validation of assay and algorithms used to estimate HIV incidence” was introduced to the audience. It was proposed that the document include: introduction, key definitions and terminology, methodology for validation of assay-based incidence estimation, characteristics of specimen sets needed for validation, specimen quality, sample size and statistical consideration for validation, calculation of assay-based incidence, technical considerations, statistical test of equivalence, and references.

The role of FDA in regulating HIV incidence assays was discussed. FDA will get involved if test results are reported to the patient; therefore, individual diagnosis will require FDA regulation. It was mentioned, however, that FDA will need some guidance from experts, likely from the WHO working group, in order to establish criteria to regulate HIV incidence assays.

There was a lot of discussion about how prototype development and assay validation should be performed and which institutions or international programs would like to have more certainty on use of validated assays based on good scientific evidence. There was consensus that WHO was in the best position, even though it is not easy to play that role.

The importance of a more realistic product profile was highlighted. The product profile may need to be more flexible in the case that one test alone may not be able to do the job, so development of algorithm may play an important role and will have to find ways to overcome shortcomings such as FRRs, CD4 data, and how to utilize data. Producers would need to provide information on how the product will be used and its potential limitations.

The importance of defining the number and type of specimens given to the developers and to an independent reference lab for evaluation was indicated. The evaluation would assess operational aspects and algorithms to reach criteria to move to another stage such as a pilot study using prototype kits. CEPHIA was mentioned as an effort to get the appropriate specimen panels for testing. WHO, with some collaborators, is already working on this document, but a statistician is needed. It was suggested that one person from each of CDC’s HIV divisions (i.e., DHAP and DGHA) and assays developers should be enlisted to help write document 1. A period of 6 months from this meeting was set to have a draft to circulate among the group. Document 1 should be ready to use for to validate some assays and be finalized by June 2012.

There was some discussion about whether Document 2 should be written. There was some confusion about this document; some people thought that the guidance for field validation has been established and others thought that the field needs to know how to validate assays.
A proposal was made to consider countries that are already performing recency testing on specimens from individuals with newly diagnosed HIV infection—a document to provide guidance on the correct use and interpretation of the data collected would be very helpful.

WHO and International programs would like more information on use of validated assays based on good scientific evidence. Some people agreed that it is a good idea to reach consensus on some parameters for the product profile, but it was not clear who will make the final decision on guidance. A guidance document will be useful to put some order on the development, evaluation, and commercialization of assays.

**Main Conclusions and Recommendations for Session 5:**

- WHO would like to have a document that includes criteria for validating assays that will be recommended for use in estimating HIV incidence. This document to provide guidance on how to validate and assay before the country implements it could serve as a bridge to provide the confidence needed to move from using the current method (BED) to using new assays.
- Document 2 would serve as a bridge between development and field application, and that it would be easy to follow during implementation and could be part of or an annex to Document 1.
- Development of a “Status report on the state of incidence testing” was proposed in lieu of developing formal recommendations.

**7.2 Session 6: Role and task of HIV incidence working group, remaining issues, and next steps**

The session opened with perspectives on HIV assays and uses. It was pointed out that the interest by assay developers/industry is fading. Although a few promising new assays were described during this meeting, and a couple have recently been commercialized, the new assays are not yet available for routine use. Difficulties may lie in the different perspectives on incidence testing by various groups involved. The role and tasks of the WHO incidence working group was presented, and objectives and achievements were discussed during the presentation.

**The main objectives set in 2008 when the HIV Incidence Working Group was first established were to:**

- Assess and foster the development of improved HIV incidence assays
- Share and advance the science on using assays to estimate HIV incidence and create a central resource/knowledge repository
- Provide guidance and advocacy documents on the use of HIV incidence assays

Reviewing the objectives and products of the working group it was agreed that most goals were achieved, but a few key products are still pending

- Validation protocol
From the statistical discussions, the group leaves with new definitions, new formulas and new calculators, and a better grasp on FRRs. But all of this information needs to be updated in the tools currently available.

There is yet a need for consensus on an ideal HIV incidence assay. Although many promising assays are being evaluated, there is a need to remember what it happened with BED and to avoid a repetition of the story.

Analyses to determine association of various factors with recent infection or to monitor the proportion and characteristics of HIV infections which are were mentioned as important points for discussion.

Since the inception of the HIV Incidence Working Group, several groups (critical path, the CEPHIA project) have been involved in the HIV incidence field. This is not seen as handicap but rather an advantage to advancing the development and validation of HIV incidence assays.

There was no statement to follow up the one released by UNAIDS in 2006 about misclassification using the BED assay. However, issuing a recommendation now will be premature since there is no assay currently available to replace BED. Because release of recommendations will require more stringent clearance by WHO, issuing a status report was seen as a better alternative.

Another suggestion was made to prepare an “Incidence Fact Sheet” instead of a status report. To reach several audiences, including national authorities, the fact sheet would be concise and not too technical, and would outline how to use the existing tools for estimating incidence. Some countries like USA, Canada, and France presented the idea of forming a subcommittee to prepare a short guide on how to use assays, because WHO’s focus is mainly on developing countries.

WHO mentioned that the budget to promote regional workshops or other incidence activities in different regions may be limited.

The inclusion of a list of participants in WHO documents was suggested.

WHO will take the lead with some meeting participants in promoting and disseminating relevant documents and continue to keep the website up to date.

**Main Conclusions and Recommendations for Session 6:**

- Post citations on WHO website of all the papers published in the few years
- Prepare a workshop to promote use of the new HIV guidelines for HIV incidence assays
Annual Meeting of the WHO Working Group on HIV Incidence Assays
Atlanta
18-24 August 2011

- Develop a status report on HIV incidence assays
- Complete the critical pathway for HIV incidence assays
- Produce a guidance document for developed countries
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<tr>
<th>Day 1: Statistical consultation (Statistics sub-committee members only)</th>
<th>Thursday, 18 August 2011</th>
<th>Presenter/Facilitator</th>
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<tbody>
<tr>
<td>08:30 – 09:00</td>
<td>Welcome, introduction, objectives and expected outcomes</td>
<td>Presenters: Txema Calleja Joyce Neal</td>
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<tr>
<td>09:00- 10:00</td>
<td>Session A: Approaches for estimating the mean duration of recency</td>
<td>Presenters: Alex Welte</td>
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<td>10:00-10:30</td>
<td>Coffee break</td>
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<tr>
<td>10:30-12:30</td>
<td>Session A (continued): Approaches for estimating mean duration of recency</td>
<td>Presenters: Anindya De Debra Hanson</td>
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<td>12:30-13:30</td>
<td>Lunch</td>
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<tr>
<td>13:30-15:00</td>
<td>Session A Methodology Discussion</td>
<td>Facilitator: Tim Green</td>
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<td>15:00-15:30</td>
<td>Coffee Break</td>
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<tr>
<td>15:30 – 17:00</td>
<td>Discussion and Recommendations</td>
<td>Facilitator: Meade Morgan</td>
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<tr>
<td>Day 2: Statistical consultation (Statistics sub-committee members only)</td>
<td>Friday, 19 August 2011</td>
<td>Presenter/Facilitator</td>
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<tr>
<td>8:30 – 9:30</td>
<td>Session B: Sample size calculators and considerations for sample sizes</td>
<td>Presenters: Alex Welte Anindya De</td>
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<td>9:30 – 10:30</td>
<td>Session B Discussion and recommendations</td>
<td>Facilitator: Lillian Lin</td>
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<td>10:30-11:00</td>
<td>Coffee Break</td>
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<td>11:00 – 12:00</td>
<td>Session C: Incidence estimation and statistical testing</td>
<td>Presenters: Alex Welte Rick Song Ron Brookmeyer</td>
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<td>12:00-13:00</td>
<td>Lunch</td>
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<tr>
<td>13:00-14:30</td>
<td>Session C: Discussion and recommendations</td>
<td>Facilitator: Tim Green</td>
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<td>14:30-14:45</td>
<td>Coffee Break</td>
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<td>14:45-16:00</td>
<td>Development of consensus statement</td>
<td>Facilitator: Meade Morgan</td>
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<tr>
<td>Time</td>
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<td>16:00</td>
<td>Adjourn</td>
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<tr>
<td>Day 3 (All)</td>
<td>Monday, 22 August 2011</td>
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<td>Registration of participants</td>
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<td>08:00 –08:30</td>
<td>Welcome remarks</td>
<td>CDC</td>
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<td>08:30 - 09:00</td>
<td>Introduction, objectives and expected outcomes, review agenda</td>
<td>Txema Calleja</td>
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<tr>
<td>09:00- 09:30</td>
<td><strong>Session 1: Overview of Incidence Assays and Incidence Estimation Methodologies: Challenges Facing the Field</strong></td>
<td>Presenters: Bharat Parekh, Andrea Kim</td>
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<tr>
<td>9:30-10:30</td>
<td><strong>Session 2: Statistical Challenges for Incidence Estimation and Report from Statistical Consultation Meeting</strong></td>
<td>TBD</td>
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<td>10:30-11:00</td>
<td>Coffee break</td>
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<tr>
<td>11:00-12:30</td>
<td><strong>Session 1 &amp; 2: Discussion</strong></td>
<td>Facilitator: Bernie Branson</td>
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<td>12:30-13:30</td>
<td>Lunch</td>
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<tr>
<td>13:30-14:30</td>
<td><strong>Session 3a: Update on specimen repositories and cross-testing initiatives</strong></td>
<td>Presenters: Gates initiative: Gary Murphy/Mike Busch/Chris Pilcher, CDC initiative: Michele Owen</td>
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<td>• CEPHIA initiative</td>
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<td>14:30-15:45</td>
<td><strong>Session 3b: Incidence Assays under Evaluation</strong></td>
<td>Bharat Parekh – LAg Avidity EIA, and two-well rIDRm Avidity Index EIA, Michele Owen – BioRad 1-2-O EIA Avidity, Sheila Keating – Ortho Vitros, Barbara Suligoi – Architect Avidity Assay</td>
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<td>• Development</td>
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<td>• Mean duration of recency determination</td>
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<td>• In-house evaluations</td>
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<td>15:45- 16:00</td>
<td>Coffee break</td>
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<tr>
<td>16:00- 17:00</td>
<td><strong>Session 3a and 3b Discussion</strong></td>
<td>Facilitator: John Nkengasong</td>
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<td>Day 4: (ALL)</td>
<td>Tuesday, 23 August 2011</td>
<td>Presenter/Facilitator</td>
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| 08:30-10:00 | **Session 4a: Tests for recent infection on the horizon**  
- Development  
- Mean duration of recency determination  
- In-house evaluations | **Presenters:**  
Kelly Curtis- Luminex  
Tim Granade - Rapid I-P Assay  
Chris Pilcher – Molecular tests  
Matthew Cousins – High resolution melting |
| 10:00-10:30 | **Session 4a Discussion** | **Facilitator: Gary Murphy** |
| 10:30-11:00 | Coffee Break | |
| 11:00-12:00 | **Session 4b: Update on new results from TRI field evaluations**  
- FRR studies  
- Field validations: cross-sectional vs prospective  
- Application | **Presenters:**  
Vietnam FRR – Nguyen Anh Tuan  
Ghana FRR – John Aberle-Grasse |
| 12:00-13:00 | Lunch | |
| 13:00 – 15:30 | **Session 4b (continued): Update on new results from TRI field evaluations**  
- FRR studies  
- Field validations: cross-sectional vs prospective  
- Application | **Presenters:**  
FHI validation and FRR (Vietnam, Ethiopia, South Africa, Mozambique) – Tim Mastro  
JHU validation and FRR in East and Southern Africa - Oliver Laeyendecker  
Kenya incidence activities - Andrea Kim/Clement Zeh  
Central America FRR and application – Gabriela Paz-Bailey  
US incidence surveillance – Buzz Prejean |
| 15:30-16:00 | Coffee Break | |
| 16:00-17:00 | **Session 4b Discussion** | **Facilitator: Joyce Neal** |
| 17:00 | Adjourn | |
### Day 5: (All)  | Wednesday, 24 August 2011 | Presenter/Facilitator
---|---|---
8:30-9:30 | **Session 5: Update on development of WHO guidance documents for HIV incidence assays**<br>**Session 5 Discussion**<br>**Coffee Break**<br>**Session 6: Role and task of HIV incidence working group, remaining issues, and next steps**<br>**Session 6 Discussion**<br>**Close Out and Adjourn** | **Presenters:**<br>Gaby Vercauteren<br>**Facilitator:** Txema Calleja<br>**Presenters:**<br>Gaby Vercauteren<br>Txema Calleja<br>**Facilitator:** Tim Mastro<br>Txema Calleja
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- Cecilia Ophelia Riano